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Microbubble potentiated transcranial duplex ultrasound enhances IV thrombolysis in acute stroke

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Abstract
Background We studied whether 2 MHz transcranial color-coded duplex ultrasound (TCCD), combined with a *second generation* ECA, accelerate IV rtPA-thrombolysis in the acute phase of MCA stroke more than TCCD monitoring alone.
Methods Non-randomized acute MCA stroke patients undergoing IV rtPA-thrombolysis and 2 MHZ-TCCD monitoring over 60 min, with ($N = 11$) or without ($N = 15$) additional continuous ECA (5 ml, SonoVue®) perfusion, were compared. Recanalization of the MCA was measured pre- and post-thrombolysis with the thrombolysis in brain ischemia (TIBI) grading system, clinical outcome was assessed at admission and 24 h after treatment using the NIH stroke scale (NIHSS).
Results Patients who received ECA improved their NIHSS significantly more than those who were only TCCD monitored (Mann–Whitney $U = 48.0$; $P = 0.050$), and their flow signal improved more (Mann–Whitney $U = 40.0$; $P < 0.03$).
Conclusions The results of this pilot study show that in IV-thrombolysis the use of ECA in addition to TCCD monitoring lead to a greater immediate clinical improvement and to a better flow signal.

Keywords Stroke · Thrombolysis · Transcranial ultrasound · Microbubbles

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Introduction

Transcranial color-coded duplex (TCCD) which visualizes basal cerebral arteries and therefore localizes precisely blood clot site is increasingly used as a diagnostic tool in the monitoring of acute stroke. Continuous application of 2 MHz TCCD with or without fibrinolytic agent has been shown to accelerate thrombolysis in acute ischemic stroke due to middle cerebral artery occlusion [1–3]. Synergistic effects between microbubbles used as contrast agents and tissue plasminogen activator (rt-PA) accelerate in vitro enzymatic fibrinolysis of clots exposed to 2-MHz pulsed-wave TCCD ultrasound [4]. Furthermore, it has been shown that continuous monitoring of acute MCA main stem occlusion using TCCD and intravenously administered galactose-based microbubbles echocontrast agent (Levovist®) in a few patients who were treated with IV rt-PA within 3 h of symptom onset is feasible but suggested a high rate of asymptomatic hemorrhagic transformation [5].

Recently, a non-randomized study used in patients with acute stroke due to MCA occlusion galactose-based microbubbles echocontrast agent (ECA) (Levovist®) during sonothrombolysis. It showed that microbubbles safely accelerate transcranial Doppler-enhanced thrombolysis in achieving a significant more complete degree of MCA recanalization and a trend toward better clinical evolution and long-term outcome [6].

It has been hypothesized that fluid motion resulting from ultrasound-induced cavitations could accelerate fibrinolysis by promoting the transport of activators and plasminogen to their target sites on fibrin within the clot [7, 8]. Furthermore it has been shown that ultrasound is able to induce reversible changes in the fibrin mesh by creating microstreams of plasma through the thrombus and accelerating the transport

and penetration of tPA into the clot, resulting in a more complete and faster clot dissolution [8].

We studied whether TCCD ultrasound monitoring combined with a *second generation*, phospholipid encapsulated sulphur hexafluoride microbubble ECA (SonoVue®), accelerates IV rtPA-thrombolysis in the acute phase of middle cerebral artery (MCA) stroke more than TCCD ultrasound monitoring alone.

Patients and methods

Patients

The patients in this non-randomized study are those who received IV-rtPA thrombolysis combined with a 60 min TCCD pulsed-wave monitoring, with and without echocontrast agent perfusion, in whom after 30 min TIBI flow grades improved by one or more grades or whose TIBI grade was ≥3 and who, therefore were not candidates for a combined IV-intraarterial thrombolysis. There were two groups, one with (11 *patients*) and one without (15 *patients*) additional continuous perfusion of an ECA. The selection criterion between the two groups was the presence of unsatisfying (which means that there was still a weak color-coded duplex signal to allow to depict but not ''to follow'' the entire vessels of the circle of Willis and the MCA in particular) temporal bone windows, prompting the use of ECA. Furthermore, B-mode visualization of the parenchyma was good enough to recognize plane of insonation (e.g. mesencephalon).

Methods

Imaging

CT-scan, in order to exclude hemorrhage, and CT-angiography were performed prior to thrombolysis in all patients. Sonographers were blinded to these exams. Control CT and/or MRI and MR angiography were performed 2–15 days later.

R-tPA thrombolysis

All stroke patients in this study were treated with 0.9 mg/Kg TPA (10% bolus) within 3 h of stroke onset.

TCCD examination pre-thrombolysis

In order to assess the exact site of occlusion in the MCA, three certified sonographers experienced in TCCD thrombolysis-monitoring in acute stroke, performed standard 2-MHz TCCD examination in all patients in the emergency room before tPA administration (Acuson, Sequoia, USA). At this point, patients with unsatisfying temporal bone windows received a bolus of 0.1 ml on each temporal side. According to previous TCCD studies [1, 5], temporal insonation in the mesencephalic axial plane and B-mode imaging was performed in all patients in order to visualize the hyperechogenic lateral fissure anterior and lateral to the cerebral peduncles. The ultrasound beam was positioned at the blood flow/thrombus interface. Main stem occlusion of the MCA was diagnosed when no flow could be demonstrated despite good visualization of the ipsilateral vessels of the circle of Willis. Residual blood flow of the MCA, when present, was assessed with an insonation depth 45–65 mm.

TCCD monitoring

Parallel to the onset of rt-PA treatment, monitoring of the residual blood flow in the affected MCA was performed in all patients and those with unsatisfying temporal bone window received additional ECA continuous infusion. Site of occlusion on the MCA was defined according to the thrombolysis in brain ischemia (TIBI) grading system. Accordingly residual flow was: TIBI 0: absent; TIBI 1: minimal; TIBI 2: blunted; TIBI 3: dampened; TIBI 4: stenotic/asymmetric; TIBI 5: normal/symmetric. Partial recanalization at 24 h was considered when the TIBI grade evolved from 0–1 to 2–3 and complete recanalization with a final TIBI grade of 4 or 5 (Fig. 1). Recanalization of the MCA was recorded by the sonographers (who were blinded to the NIHSS score) using the TIBI grading system post-thrombolysis. When there was no residual Doppler signal, the position of the ultrasound beam was verified every 5 min using B-mode imaging. During recanalization

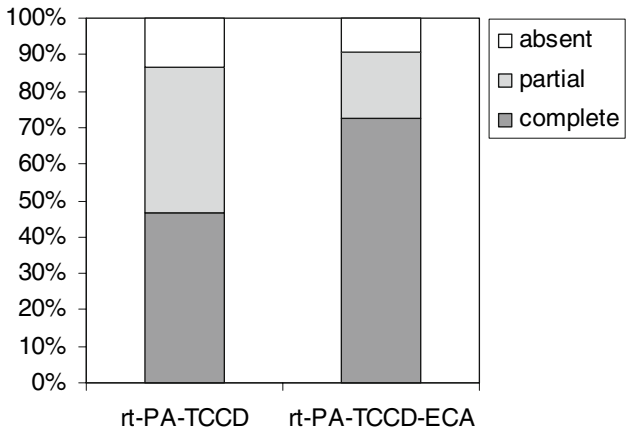

Fig. 1 Percentage of degree of recanalization of the MCA (middle cerebral artery) according to TIBI (thrombolysis in brain ischemia) criteria after rtPA thrombolysis in group 1 (rtPA-TCCD) and 2 (rtPA-TCCD-ECA)

process the ultrasound beam was shifted to maintain it at the blood flow-thrombus interface. All patients were monitored with continuous hand-held 2-MHz TCCD (Pulsed-wave mode power output: 189 mW/cm^2 (data from the manufacturer); sample volume 10 mm; Acuson, Sequoia, USA) over 60 min. Gain settings, transmit power, pulse repetition frequency were adapted individually for an optimal signal-to-noise ratio. These parameters remained unchanged during the entire monitoring.

Echocontrast agent

In accordance to the contraindications as stated by EMEA (European Agency for the Evaluation of Medicinal Products; London 2004), 11 patients with unsatisfying temporal bone windows received one bolus injection of 0.1 ml on each temporal side, in order to assess the exact site of occlusion in the MCA before TCCD monitoring. This procedure was repeated for control examination at 24 h. During TCCD monitoring these 11 patients received a continuous infusion in the cubital vein of a total dose of 5 ml of microbubble phospholipid encapsulated sulphur hexafluoride echocontrast agent (8 µl/45 µg/ml) over 60 min (SonoVue®, Bracco SA, Switzerland).

Clinical outcome

Clinical severity of stroke was assessed in all the patients using the NIH Stroke Scale (NIHSS) at admission and at 24 h after treatment by a clinician who was blinded to the ultrasonographic monitoring modality. Clinical improvement was considered when the NIHSS score improved at 24 h of at least ≥4 points.

Results

A total of 15 patients (*group* 1) (9 men, 6 women; mean age 65.1 years) underwent IV thrombolysis and TCCD monitoring over 60 min. 12 patients (75%) had a NIHSS score ≥10 at admission.

A total of 11 patients (*group* 2) (6 men, 5 women; mean age 68.9 years) were treated with IV thrombolysis and received additional ECA enhanced TCCD monitoring over 60 min. 7 patients (63.6%) had a NIHSS score ≥10 at admission (Table 1).

Age, sex, IV-thrombolysis mean time at onset, and NIHSS score at admission, and initial TIBI grades did not differ significantly between the two groups.

Residual blood flow in the MCA was evaluated with the TIBI score pre-thrombolysis, during thrombolysis (30 min), post thrombolysis (60 min) and at 24 h. 18 patients had a TIBI score pre-thrombolysis <3 (10/15 in group 1 and 8/11 in group 2). The flow signal (measured according to the improvement of TIBI grades) improved more in group 2 than in group 1 after 30 min (Mann–Whitney $U = 40.5$; $P < 0.03$) and remained significant after 60 min (Mann–Whitney $U = 40.0$; $P < 0.03$). Complete recanalization after 1-h systemic thrombolysis occurred in 8 patients in group 1 (median time: 42.25 min) and in 7 patients in group 2 (median time: 32.85 min). 2 patients suffered from symptomatic hemorrhagic transformation after treatment: 1 in group 1 (rtPA + TCCD) and 1 in group 2 (rtPA + TCCD + ECA). There was no significant difference in the bleeding rate between the two groups. Group 2 improved the NIHSS significantly more than group 1 (Mann–Whitney $U = 48.0$; $P = 0.05$).

Discussion

There are two main results of the present non-randomized pilot study. (1) ECA enhanced TCCD monitored rtPA thrombolysis is superior to TCCD monitored rtPA thrombolysis in terms of residual flow improvement as measured by TIB, especially during the first 30 min. (2) It is also superior in the immediate (24 h) clinical improvement as measured by NIHSS. Last but not least, the rate of hemorrhagic transformation was not different between the two groups.

The present study thus confirms with a different echocontrast agent (second generation ECA SonoVue® versus Levovist®), a different transcranial ultrasound technique (TCCD versus TCD), and different durations of insonation (1 vs. 2 h), the results of a recent study showing superior recanalization by using echocontrast agents in addition to Doppler monitoring of thrombolysis [6]. Though the exact mechanisms on the effects of combined TCCD and microbubbles remain unknown in humans, several experimental studies have demonstrated the ability of ultrasound routinely used for transcranial diagnostic applications to potentiate enzymatic thrombolysis [7–11]. Clinical studies have confirmed these effects in acute stroke patients [2, 12–14]. Moreover, the streaming of microbubbles contained in echocontrast agents driven by ultrasound energy seems to lead to mechanical damage on the surface of the thrombus. This increases infiltration of tPA in the thrombus and thus further accelerates clot dissolution by lowering the energy needed for cavitation and this even in the absence of thrombolytic drugs [1, 15–19]. However, there are actually no data demonstrating that cavitations take place in the human brain using diagnostic echocontrast enhanced TCD/TCCD monitoring. The in vivo stability of the echocontrast agents is related to their shell characteristics and to the solubility of the gas used in their preparation [20]. Second generation echocontrast agents such as SonoVue® which

Table 1 TIBI gains, clinical improvement and symptomatic hemorrhagic transformations are summarized for all patients

Patients	NIHSS initial	NIHSS improvement	Occlusion site	TIBI gain at 30 min	TIBI gain at 60 min	Symptomatic hemorrhagic transformation
IV rtPA-TCCD						
1.	11	Yes	Proximal MCA	2	2	No
2.	6	Yes	Distal MCA	1	1	No
3.	17	Yes	Distal MCA	1	1	No
4.	20	Yes	Proximal MCA	1	2	No
5.	15	No	Proximal MCA	0	0	No
6.	14	No	Distal MCA	1	1	No
7.	13	No	Distal MCA	2	2	No
8.	16	No	Distal MCA	2	2	Yes
9.	15	No	Proximal MCA	0	0	No
10.	17	Yes	Distal MCA	1	3	No
11.	15	No	Distal ICA + proximal MCA	0	0	No
12.	7	Yes	Distal MCA	2	2	No
13.	14	No	Distal MCA	1	2	No
14.	13	No	Distal MCA	1	1	No
15.	9	No	Distal MCA	2	2	No
IV rtPA-TCCD-ECA						
1.	15	Yes	Distal MCA	2	2	No
2.	9	Yes	Distal MCA	3	4	No
3.	11	No	Distal ICA + proximal MCA	1	1	Yes
4.	17	Yes	Distal MCA	2	3	No
5.	17	Yes	Distal ICA + proximal MCA	1	3	No
6.	9	Yes	Distal MCA	2	2	No
7.	19	Yes	Proximal MCA	2	2	No
8.	8	Yes	Proximal MCA	2	3	No
9.	9	Yes	Distal MCA	2	2	No
10.	14	No	Proximal MCA	1	1	No
11.	12	Yes	Distal MCA	3	3	No

TCCD = Transcranial color-coded Doppler; ECA = echocontrast agent; TIBI = Thrombolysis in brain ischemia; MCA = middle cerebral artery; ICA = internal carotid artery

contain poorly soluble gases such as sulphur hexafluoride agents increase the accuracy in detection of abnormalities in cerebral arteries by improving the Doppler signal to noise ratio. They also have a longer action time than air-containing agents like Levovist® [20]. Moreover, they contain microbubbles with a much smaller diameter than air-filled microbubbles which improves the passage of the pulmonary capillary bed [20].

It has been shown in angiographic studies [21, 22] that IV rtPA is relatively ineffective with a rate of early recanalization of approximately 25% in patients with large vessel occlusion such as MCA main stem occlusion. There is thus a need for additional therapies [23]. Our study has limitations, it is non-randomized and touches a relatively small sample size of patients who responded to IV thrombolysis within 30 min of infusion, but it lends support to the combined use of Doppler monitoring and echocontrast agents.

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